METHODS AND COMPOSITIONS FOR TREATMENT OF OCULAR NEOVASCULARIZATION AND NEURAL INJURY

This application claims priority pursuant to 35 USC 119 to provisional application Serial No.

5   60/244,850, filed Nov. 1, 2000.

Background Of The Invention

Loss of visual acuity is a common problem associated with aging and with various conditions of

10 the eye. Particularly troublesome is the development of unwanted neovascularization in the cornea, retina or choroid. Choroidal neovascularization leads to hemorrhage and fibrosis, with resultant visual loss in a number of recognized eye diseases, including macular

15 degeneration, ocular histoplasmosis syndrome, myopia, diabetic retinopathy and inflammatory diseases.

Age-related macular degeneration (AMD) is the leading cause of new blindness in the elderly, and choroidal neovascularization is responsible for 80% of

20 the severe visual loss in patients with this disease. Although the natural history of the disease is eventual quiescence and regression of the neovascularization process, this usually occurs at the cost of sub-retinal fibrosis and vision loss.

25 Traditional treatment of AMD relies on occlusion of the blood vessels using laser photocoagulation. However, such treatment requires thermal destruction of the neovascular tissue, and is accompanied by full-thickness retinal damage, as well as damage to medium

30 and large choroidal vessels. Further, the subject is left with an atrophic scar and visual scotoma.

Moreover, recurrences are common, and visual prognosis is poor.

Recently, forms of photocoagulation have been devised which attempt to reduce the damage attendant to traditional photocoagulation. For example, transpupillary thermotherapy (TTT) utilizes a low intensity laser in combination with a large "spot" (irradiation focal point) size and long exposure to close choroidal neovascularization and thereby treat macular degeneration. This procedure is said to reduce the amount of secondary damage seen in the use of traditional photocoagulation procedures.

Photodynamic Therapy

Recent research in the treatment of neovascularization have had the aim of causing more selective closure of the blood vessels, in order to preserve the overlying neurosensory retina. One such strategy is a treatment termed photodynamic therapy or PDT, which relies on low intensity light exposure of photosensitized tissues to produce lesions in the newly developing blood vessels. In PDT, photoactive compounds are administered and allowed to reach a particular undesired tissue which is then irradiated with a light absorbed by the photoactive compound. This results in destruction or impairment of the tissue immediately surrounding the locus of the photoactive compound without the more extensive ocular tissue damage seen when photocoagulation is used.

Photodynamic therapy of conditions in the eye has been attempted over the past several decades using various photoactive compounds, e.g., porphyrin derivatives, such as hematoporphyrin derivative and

5   Photofrin porfimer sodium; "green porphyrins", such as benzoporphyrin derivative (BPD), MA; and phthalocyanines. Photodynamic treatment of eye conditions has been reported to actually enhance the visual acuity of certain subjects in some

10   circumstances. U.S. Patent No. 5,756,541.

However, although generally more safe than photocoagulation, there are certain dangers involved in performing PDT. For example, low intensity lasers in conjunction with the systemic injection of

15   vertporfin is currently the only approved PDT for treatment of age-related macular degeneration. But studies have shown that the use of vertporfin at high doses (12 and 18 mg/m^2) result in long term or permanent scarring of the retina, chronic absence of

20   photoreceptor cells, and optic nerve atrophy. Reinke et al., *Ophthalmology* 106:1915 (October 1999), incorporated by reference herein. At lower concentrations of vertporfin (e.g., about 6 mg/m^2) PDT is effective to slow vascular outgrowth and associated

25   edema somewhat, but treatment appears to be necessary every few months.

Additionally, while PDT is clearly efficacious in some patients, this mode of treatment has resulted in a lower percentage of patients reporting an increase

30   in visual acuity, or a halting in the progression of visual deterioration, than would be expected

theoretically. The reasons for this have not been
clearly understood in the literature, but may relate
to PDT-induced neurosensory damage which limits
efficacy.

5

The Alpha Adrenergic Receptors

Human adrenergic receptors are integral membrane
proteins which have been classified into two broad

10   classes, the alpha and the beta adrenergic receptors.
Both types mediate the action of the peripheral
sympathetic nervous system upon binding of
catecholamines, norepinephrine and epinephrine.
Norepinephrine is produced by adrenergic nerve

15   endings, while epinephrine is produced by the adrenal
medulla. The binding affinity of adrenergic receptors
for these compounds forms one basis of the
classification: alpha receptors tend to bind
norepinephrine more strongly than epinephrine and much

20   more strongly than the synthetic compound
isoproterenol. The preferred binding affinity of these
hormones is reversed for the beta receptors. In many
tissues, the functional responses, such as smooth
muscle contraction, induced by alpha receptor

25   activation are opposed to responses induced by beta
receptor binding.
Subsequently, the functional distinction between
alpha and beta receptors was further highlighted and
refined by the pharmacological characterization of

30   these receptors from various animal and tissue
sources. As a result, alpha and beta adrenergic

receptors were further subdivided into α_1, α_2, β_1, and β_2 subtypes.

Functional differences between α_1 and α_2 receptors have been recognized, and compounds which

5 exhibit selective binding between these two subtypes have been developed. Thus, in WO 92/0073, the selective ability of the R(+) enantiomer of terazosin to selectively bind to adrenergic receptors of the α_1 subtype was reported. The α_1/α_2 selectivity of this

10 compound was disclosed as being significant because agonist stimulation of the α_2 receptors was said to inhibit secretion of epinephrine and norepinephrine, while antagonism of the α_2 receptor was said to increase secretion of these hormones. Thus, the use of

15 non-selective alpha-adrenergic blockers, such as phenoxybenzamine and phentolamine, was said to be limited by their induction, through the α_2 adrenergic receptors, of increased concentrations of plasma catecholamine and attendant physiological sequelae

20 (increased heart rate and smooth muscle contraction).

For a general background on the α-adrenergic receptors, the reader's attention is directed to Robert R. Ruffolo, Jr., α-Adrenoreceptors: Molecular Biology, Biochemistry and Pharmacology, (Progress in

25 Basic and Clinical Pharmacology series, Karger, 1991), incorporated by reference herein, in which the basis of α_1/α_2 subclassification, the molecular biology, signal transduction, agonist structure-activity relationships, receptor functions, and therapeutic

applications for compounds exhibiting α-adrenergic receptor affinity is explored.

The cloning, sequencing and expression of alpha receptor subtypes from animal tissues has led to the

5   subclassification of the α_1 adrenoreceptors into the further classifications of α_{1A}, α_{1B}, and α_{1D}. Similarly, the α_2 adrenoreceptors have also been classified α_{2A}, α_{2B}, and α_{2C} receptors. Each α_2 receptor subtype appears to exhibit its own pharmacological and tissue

10   specificities. Compounds having a degree of specificity for one or more of these subtypes may be more specific therapeutic agents for a given indication than the currently employed α_2 receptor pan-agonists (such as the drugs clonidine and

15   brimonidine).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows that brimonidine inhibits the

20   post PDT decrease in retinal functionality as compared to a saline control.

Figure 1B shows that brimonidine does not affect the area of ablation of CNV following PDT.

Figure 2B shows that Alphagan® (brimonidine) has

25   no effect on retinal thickness in non-PDT-treated rabbit eyes.

Figure 2B shows that brimonidine decreases the increase in retinal thickness (edema) appearing after PDT treatment.

30

SUMMARY OF THE INVENTION

The present invention concerns compositions and
methods for the treatment of ocular

5   neovascularization, including the reduction of
neurosensory function, which is attendant to current
therapies such as photocoagulation and PDT. In a
preferred aspect, the invention is drawn to an
improved method of performing photodynamic therapy

10   comprising treating the patient with an effective
amount of a neuroprotective agent, selected from alpha
adrenergic receptor modulators. Preferably, the alpha
adrenergic receptor modulator is an alpha adrenergic
receptor agonist. In a further preferred embodiment,

15   the alpha receptor agonist is an alpha 2 receptor
agonist, even more preferably an alpha 2B and/or an
alpha 2C agonist. It is preferred that these agents
be at least selective, and even specific, for the
indicated receptors or receptor subtypes.

20   The present invention is also directed to a
method for reducing or eliminating a decrease in
neurosensory retinal function following laser
treatment of chorodial neovanscularization (CNV)
while maintaining the vascular occlusion therapeutic

25   effect of such therapy, the method comprising the
steps: a) administering to a mammal having a CNV a
therapeutically effective amount of an alpha receptor
agonist, b) subjecting said mammal to laser
irradiation of the retinal locus of the CNV; wherein

30   the amount of neurosensory retinal function following
steps a) and b) is greater than when said mammal is

subjected to step b) without step a). In a preferred
embodiment, the mammal is given a therapeutically
effective amount of a pharmaceutically acceptable
photoactivated dye capable of accumulation in the
5 locus of a choroidal neovascularization and destroying
tissue when exposed to light of the same wavelength as
the laser.

In these methods, the alpha adrenergic receptor agonist is preferably an alpha 2 selective agonist, even more preferably an alpha 2B and/or alpha 2C selective agonist, most preferably, an alpha 2B

5   selective agonist.   In one preferred embodiment the alpha 2 selective agonist is selected from brimonidine and clonidine.

Other alpha 2B selective compounds include AGN 960, AGN 795 and AGN 923. The structure of AGN 960 is

10   presented elsewhere in this patent application. The structure of AGN 795 is as follows:
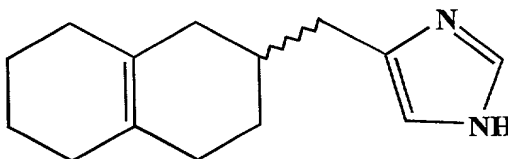
15   The structure of AGN 923 is as follows:
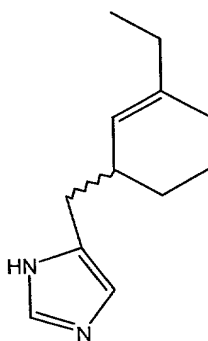
20   By "effective amount" of a neuroprotective agent (such as an alpha adrenergic agonist) is meant an

amount effective to reduce the amount of cell death among the neurons of the retina and optic nerve (e.g., photoreceptors, retinal ganglion cells, and bipolar cells, or any of these) caused by the photoactive

5   component of laser treatment as compared to a similarly situated CNV patient receiving laser treatment who does not receive treatment with the neuroprotective agent.

In another embodiment, the invention is drawn to

10   an improved method of performing photodynamic or photocoagulation therapy comprising treating the patient with an effective amount of an agent effective to protect the neurons of the retina and optic nerve (e.g., photoreceptors) from damage caused by laser

15   irradiation or the photoactive component of PDT treatment (neuroprotective agent).

An additional benefit of this therapy is the resultant reduction in edema and extravascularization of fluid that laser treatment causes.

20   By "effective amount" of a neuroprotective agent is meant an amount of such agent effective to reduce the extent to which, or the rate at which, new blood vessels are formed in the retina of a CNV patient as compared to a similarly situated CNV patient not given

25   the neuroprotective agent.

In a third embodiment, the invention is directed to an improved method of performing photodynamic therapy comprising treating the patient with an effective amount of a neuroprotective agent, and

30   irradiating the CNV with laser light sufficient to directly or indirectly cause destruction of the CNV.

In another preferred aspect, the invention is drawn to an improved method of performing photodynamic therapy comprising treating the patient with an amount of a neuroprotective agent protect neural cells so as

5   to thereby increase the amount of time necessary between PDT treatments and to slow the progression of ARMD and other ocular conditions in which neovascularization plays a part (for example ocular hiostoplasmosis syndrome (OHS) and pathogenic myopia)

10   beyond that obtained by PDT or photocoagulation alone.

When an alpha adrenergic agonist or another agent having neuroprotective activity is used in conjunction with PDT or photocoagulation, it is preferred that the amount of such agent administered to the patient is an

15   effective neuroprotective dose.

Determining the absolute dosage of the neuroprotective agent depends upon a number of factors, including the means of administration and delivery and the form of the drug. For intraocular

20   delivery agent, such as by intravitreal or subretinal injection, dosages are preferably in the range of about 0.1 ug to about 100 ug per eye; more preferably in the range of about 0.20 ug to about 50 ug per eye; even more preferably in the range of about 0.5 ug to

25   about 10 ug per eye.

The neuroprotective agent may be delivered by any means effective to expose the retinal and optic nerve cells to the agent. Thus, such agents may be delivered systemically, such as by intravenous,

30   intramuscular, or subcutaneous injection, or by oral delivery. Alternatively, the neuroprotective and/or

neovascularization-inhibiting agent(s) may be
delivered by direct injection into the eye, such as
into the anterior chamber, posterior chamber or
vitreous chamber, or by subretinal injection. The
5   reagent may also be delivered topically to the ocular
surface.

Another delivery method provides for sustained
delivery of the noeuroprotective agent using an
intraocular implant. Such implants may be, for
10   example, a biodegradable and/or biocompatible implant
or insert such as the ocular implants and inserts
disclosed in United States Patents No. 5,443,505,
5,824,072, 5,766,242; 4,853,224; 4,997,652; 5,164,188;
5,632,984; and 5,869,079, incorporated by reference
15   herein. Such implants may be inserted into a chamber
of the eye, such as the anterior, posterior or
anterior chambers, or may be implanted in the sclera,
transchoroidal space, or an avascularized region
exterior to the vitreous.
20

DETAILED DESCRIPTION OF THE INVENTION

The present invention is drawn to therapeutic
25   methods and compositions for the treatment of
intraocular neovascularization associated with
conditions such as age-related macular degeneration
(ARMD), diabetic retinopathy, ocular histoplasmosis
syndrome, and pathologic myopia.
30   The invention is more particularly concerned with
therapeutic methods combining retinal photocoagulation

or photodynamic therapy (PDT) with a neuroprotectant
agent, preferably with an alpha adrenergic agonist.
In a preferred embodiment, the agent is an alpha 2
selective agonist; even more preferably an alpha 2B

5   and/or alpha 2C selective agonist. One can also
combine the neuroprotective alpha adrenergic agonist
with other neuroprotective agents to take advantage of
different mechanistic means of providing
neuroprotection.

10   In a preferred aspect of this embodiment of the
invention, the neuroprotective agent is administered
to the patient sufficiently prior to photcoagulation
(such as TTT) or PDT treatment so as to be available
to protect nerve cells upon the commencement of

15   therapy. In another aspect of the invention, the
alpha adrenergic receptor agonist is administered a
sufficient time following photocoagulation or PDT
treatment to forestall nerve death due to such
treatment.

20   Such methods are applicable to any
photocoagulation method or to PDT treatment which
makes use of any photoactive compound. Such
photoactive compounds may include derivatives of
hematoporphyrin, as described in U.S. Patents No.

25   5,028,621; 4,866,168; 4,649,151; and 5,438,071.
pheophorbides are described in U.S.Pat. Nos.
5,198,460; 5,002,962; and 5,093,349; bacteriochlorins
in U.S. Pat. Nos. 5,171,741 and 5,173,504; dimers and
trimers of hematoporphyrins in U.S. Pat. Nos,

30   4,968,715 and 5,190,966. Other possible photoactive
compounds include purpurins, merocyanines and

porphycenes.   All of the aforementioned patents are
incorporated by reference herein.   Of course, mixtures
of photoactive compounds may be used in conjunction
with each other.

5   A currently preferred photoactive compound is
verteporfin (liposomal benzoporphyrin derivative).
This compound is currently the only photoactive agent
approved by the U.S. Food and Drug Administration for
treatment of choroidal neovascularization in
10   conjunction with photodynamic therapy.

The photoactive agent is formulated so as to
provide an effective concentration to the target
ocular tissue. The photoactive agent may be coupled to
a specific binding ligand which may bind to a specific
15   surface component of the target ocular tissue, such as
a cell surface receptor or, if desired, may be
formulated with a carrier that delivers higher
concentrations of the photoactive agent to the target
tissue. Exemplary ligands may be receptor antagonists
20   or a variable region of an immunoglobulin molecule.

The nature of the formulation will depend in part
on the mode of administration and on the nature of the
photoactive agent selected. Any pharmaceutically
acceptable excipient, or combination thereof,
25   appropriate to and compatible with the particular
photoactive compound may be used, Thus, the
photoactive compound may be administered as an aqueous
composition, as a transmucosal or transdermal
composition, or in an oral formulation. The
30   formulation may also include liposomes. Liposomal
compositions are particularly preferred especially

where the photoactive agent is a green porphyrin.
Liposomal formulations are believed to deliver the
green porphyrin with a measure of selectivity to the
low-density lipoprotein component of plasma which, in
5   turn acts as a carrier to deliver the active
ingredient more effectively to the desired site.
Increased numbers of LDL receptors have been shown to
be associated with neovascularization, and by
increasing the partitioning of the green porphyrin
10   into the lipoprotein phase of the blood, it appears to
be delivered more efficiently to neovasculature.

Consistent with the chosen formulation, the
photoactive compound may be delivered in a variety of
ways. For example, delivery may be oral, peritoneal,
15   rectal, or topical (e.g., by installation directly
into the eye). Alternatively, delivery may be by
intravenous, intramuscular or subcutaneous injection.

The dosage of the photoactive compound may vary,
according to the activity of the specific compound(s)
20   chosen, the formulation, and whether the compound is
joined to a carrier and thus targeted to a specific
tissue as described above. When using green
porphyrins, dosages are usually in the range of 0.1-50
mg/M^2 of body surface area; more preferably from about
25   1-10 mg/M^2 or from about 2-8 mg/M^2. Obviously,
parameters to be considered when determining the
dosage include the duration and wavelength of the
light irradiation, the nature of the photochemical
reaction induced by the light irradiation, and the
30   dye-to-laser time period.

Light irradiation is performed a sufficient time after the administration of the photoactive compound so as to permit the compound to reach its target tissue. Upon being irradiated with the wavelength(s)

5 appropriate to the compound(s) chosen, the compound enters an excited state and is thought to interact with other compounds to form highly reactive intermediates which can then destroy the target endothelial tissue, causing platelet aggregation and

10 thrombosis. Fluence of the irradiation may vary depending on factors such as the depth of tissue to be treated and the tissue type – generally it is between about 25 and about 200 Joules/cm^2. Irradiance typically is between about 150 and about 900 mW/cm^2,

15 but can also vary somewhat from this range.

Typically, light treatment is given about 5 minutes following administration of the photoactive drug. In a preferred embodiment, the photoactive drug is administered intravenously.

20 The other component(s) of the methods and composition of the present invention are a neuroprotective alpha adrenergic agent. Exemplary neuroprotective agents are, without exception, alpha 2 adrenergic pan-agonists such as brimonidine and

25 clonidine, and alpha 2B and or alpha 2C selective agonists such as, without limitation, those mentioned in US Patent No. 6,313,172 and patent publications WO0178703, WO0178702, WO0100586 and WO9928300. These patents and patent applications are owned by the

30 assignee of the present patent application, and are hereby incorporated by reference herein.

        In a preferred aspect of the invention a
neuroprotective agent comprising an alpha adrenergic
agonist is administered to the eye to protect it
during and after PDT treatment.   In an even more
5   preferred embodiment of the invention, the
neuroprotective agent is an alpha 2 selective agonist.
    In a most preferred embodiment of the invention, the
compound is an alpha 2B selective agonist.
        Brimonidine has been reported to have
10 neuroprotective activity.   Hence, US Patent No.
5,856,329 and Yoles E., et al., *Invest. Ophthalmol.*
Vis. Sci. 40: 65 (1999)disclose this property of
brimonidine; however, neither of these references
makes any suggestion that brimonidine be used in the
15 treatment of CNV.

        The neuroprotective alpha adrenergic agent(s) of
the present invention are delivered in any manner in
which it is effective to protect neurons and/or
inhibit neovascularization incident to
20 photocoagulation or PDT treatment.   Generally, the
agent(s) is administered prior to laser treatment, so
as to permit it to reach the ocular neural tissue
before phototherapy.   This will permit the agent(s) to
have an immediate protective effect on neural cells.
25 However, the neovascularization-inhibiting benefits of
such an agent can be realized even when given
simultaneously with, or shortly after PDT treatment.

        Additionally, the alpha adrenergic agents useful
30 in the present method may be joined, in a manner

similar to that of the photoactive compounds, to cell
surface targeting ligands, such as portions of an
antibody or immunologically active fragments to aid in
targeting the drug to ocular cells, such as the optic
5   nerve neurons and photoreceptors.

The neuroprotective alpha agonist agent may be
formulated for oral delivery in, for example, a
capsule, tablet or liquid, or for intravenous,
intramuscular, or subcutaneous injection. In such a
10   formulation, any suitable excipient may be added to
such a formulation to stabilize the active ingredient
and, particularly in the case of intravenous
administration, to provide the necessary electrolyte
balance. The alpha adrenergic agonists used in the
15   methods of the present invention may also be
formulated as a suppository or otherwise administered
rectally. Formulations appropriate for rectal drug
administration are well-known to those of skill in the
art.

20   In yet another embodiment the alpha adrenergic
agonist agents may be formulated within liposomes.
The liposomes are then able to fuse with a cell
membrane, thus delivering the nucleic acid to
receptors located at the cell surface and within the
25   cell.

As indicated above, the neuroprotective agents to
be used in the present invention may be administered
by systemic delivery, as by intravenous, intramuscular
or subcutaneous injection. In addition, these agents
30   may be delivered directly to the eye by biocompatable
and/or biodegradable implants or inserts (such as

those described in patents cited and incorporated by reference above) containing the drug , or by direct injection into the eye, for example by intravitreal and/or subretinal injection. Alternatively, the alpha

5   adrenergic agents may be topically applied to the surface in an drop.

The therapeutically effective dosage of such agents will depend upon factors including the mode of delivery, the specific activity of the polypeptide,

10   and the formulation in which the agent is fabricated.

Once a formulation and route of administration is decided upon, determining a therapeutically effective dose is routine in the pharmaceutical arts, and can be readily determined without undue experimentation using

15   suitable animal models such as, without limitation, non-human primates and rabbits.

Preferably, the dosage regimen of the neuroprotective agent will be such to permit the active aqent to remain in contact with retinal cells

20   throughout the treatment period. Thus, the agent may be administered, for example, once or twice a day for 12 weeks.

Example 1

25

A 74 year old patient presents with "wet" age-related macular degeneration (ARMD) in the foveal region of the right eye, and his condition is found to be suitable for photodynamic therapy (PDT). For one

30   week prior to the date of scheduled treatment, the

patient is given a topical dosage of brimonidine twice a day in a standard formulation.

The day of scheduled PDT treatment, the patient is administered 6 mg/M^2 of verteporfin. Fifteen
5 minutes after the start of the infusion, the patient is administered Irradiance of 600 mW/cm^2 and total fluence of 50 Joules/cm^2 from an Argon light laser.

Brimonidine administration is continued every two days throughout the 12 week evaluation period.
10 Evaluation of neural health is assayed 1 week, 4 weeks, and 12 weeks following treatment by visual inspection of the retina and test of visual acuity. The affected areas of the retina appear healthy with no whitening (indicating lack of discernable retina
15 damage) or edema one week following PDT treatment; this trend continues throughout the monitoring period. Fluorescein angiography at same time points shows minimal leakage in the treated tissue after one week, and this minimal leakage continues throughout the
20 monitoring period. No evidence of renewed neovascularization can be seen 12 weeks following PDT treatment. Additionally, no evidence of optic nerve axon loss can been seen. Tests of visual acuity 4 and 12 weeks following combined PDT and PEDF treatment
25 show no discernable loss of vision, as a result of the treatment.

Example 2

30 Same facts as in Example 1, except that rather than being given topical brimonidine, the affected eye

is given an biodegradable intraocular implant, by
injection into the vitreous humor. The implant placed
in the eye by intravitreal injection three days prior
to PDT treatment ; and is readministered 10 days
5   following PDT treatment. The retina is examined
following the 12 week evaluation period.

Evaluation of neural health is assayed 1 week, 4
weeks, and 12 weeks following treatment by visual
inspection of the retina and test of visual acuity.
10   The affected areas of the retina appear healthy with
no whitening (indicating lack of discernable retina
damage) one week following PDT treatment; this trend
continues throughout the monitoring period.
Additionally, no evidence of optic nerve axon loss can
15   been seen. Tests of visual acuity 4 and 12 weeks
following combined PDT and PEDF treatment show no
discernable loss of vision as a result of the
treatment.

20   Example 3

Brown Norway rats weighing 200-400 grams were treated
intraperitoneally (i.p.) with either brimonidine, AGN
199960, or the saline vehicle. One hour later, the
25   rats were unilaterally treated with PDT and evaluated
by electroretinography 3-4 hours later. There were 4
rats in each treatment group.

PDT was conducted as follows: Mydriasis was
induced in one eye of each rat with a drop of 0.5%
30   tropicamide. Rats were then anesthetized with
isoflurane and placed on a platform in front of a slit

lamp coupled to a Coherent diode laser for verteporfin

PDT (689 nm). Verteporfin was injected intravenously

at a dose of 6 mg/m^2. One minute later, the retina of

one eye was irradiated with a 3 mm size spot at 50

5   J/cm^2, 600 mW/cm^2 in the superior hemisphere above the

optic disk. Three to four hours later, the treated

animals were again dilated and anesthetized, then

electroretinograms (ERGs) were evaluated in each eye.

        ERGs are collected non-invasively by measuring

10   mass-cell response arising due to retinal activity

proceeding from a light stimulus. The first cells

stimulated by a flash light stimulation are the

photoreceptors at the outer retinal layer. This

response is measured as an a-wave. As the signal is

15   transduced to inner retinal neurons, a b-wave is

produced. The a-wave reflects activity in the

photoreceptors and the b-wave reflects activity in

both photoreceptors and bipolar cells.

        Before being subjected to the ERG test, the rats

20   were placed in the dark (dark-adapted) for 15 minutes.

Two types of ERG apparatus were used to generate the

results below. In the initial brimonidine experiment,

each eye was evaluated separately. A Grass

photostimulator was placed 10 cm from the recording

25   eye and flashed a single white flash lasting 10

microseconds. A gold corneal ring electrode with a

reference electrode attached to the lower eyelid

detected retinal responses. For the AGN 199960

experiments, a Ganzfeld dome was used to generate a

30   single flash and sensitive bipolar corneal electrodes

were placed on both eyes, thus allowing simultaneous

ERGs to be done. A cushioning agent on the cornea
(methylcellulose) was used in each case, and the
responses were amplified and stored in a computer.
The results reported are a-wave amplitudes, which
5   appear to be the ERG parameter most affected in
clinical PDT. As can be seen below, the loss in
retinal function induced by PDT was inhibited by an
alpha-2 receptor pan agonist (brimonidine) and a
selective alpha-2B receptor agonist (AGN 960).
10   AGN 960 has the following structure:
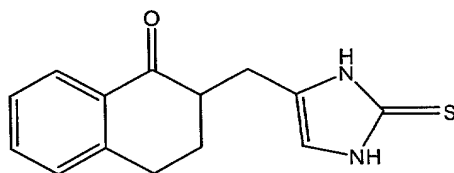
Brimonidine has the following structure:

15
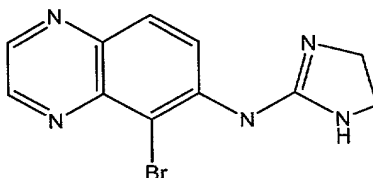
Agent	Dose	N	A Wave Amplitude (uV)		% Control
			PDT Eye	Control Eye	
Vehicle	1 ml/kg	4	70 ± 18	104 ± 17	67 ± 12
Brimonidine	1000 ug/kg	4	90 ± 15	91 ± 21	103 ± 8 *
Vehicle	1 ml/kg	4	120 ± 12	228 ± 17	53 ± 3
AGN 960	300	4	212 ± 16	218 ± 29	101 ± 13

ug/kg	*

Table 1. A-wave reflects photoreceptor function. Animals were treated with the indicated agent or vehicle i.p. 1 hour prior to PDT. * = p ≤ 0.05, comparison between drug and vehicle-treated animals utilizing an unpaired Student's t-test

Example 4

Seven pigmented rabbits were dosed with either 0.5 mls of 0.2% brimonidine (alphagan) or saline administered retrobulbarly in 1 eye of each rabbit. One hour later, the animals were treated with a 10 minute intravenous infusion of 0.2 mg/kg verteporfin, then the same eye was irradiated 10 minutes later in the lower fundus with a 689 nm diode laser at 50 J/cm^2, 600 mW/cm^2 and a spot size of 1.5 mm.

Treated eyes were then imaged by ocular coherence tomography; this method gives a measure of retinal thickness at the following time points after PDT (hours): 4, 24, 48, 72. Data are presented in Figure 1A and 1B; and show that brimonidine reduced the increase in retinal thickness (subretinal cyst + retina) in the lesion produced by PDT.

These examples illustrate certain embodiments of the present invention; however, it will be understood that the invention is solely defined by the claims that conclude this specification.